

=>

=> dhis

DHIS IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.

For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> d his

(FILE 'HOME' ENTERED AT 09:30:00 ON 10 MAR 2006)

FILE 'DISSABS, IMOBILITY, AGRICOLA, AQUASCI, BIOTECHNO, COMPENDEX,
COMPUAB, CONF, CONFSCI, ELCOM, HEALSAFE, IMSDRUGCONF, LIFESCI, OCEAN,
PAPERCHEM2, PASCAL, POLLUAB, SOLIDSTATE, ADISCTI, ADISINSIGHT, ADISNEWS,
ANABSTR, ANTE, AQUALINE, BIOENG, BIOSIS, ...' ENTERED AT 09:30:19 ON 10
MAR 2006

L1 850247 S (VLA1 OR (VLA (A) 1) OR ALPHA1 OR (ALPHA (A) 1) OR (VERY LATE
L2 28371 S L1 (S) (INFLAMMATION OR (RENAL (A) FIBROSIS) OR KIDNEY)
L3 5412 S L2 (S) (VIVO OR ADMINISTER? OR INJECT?)

FILE 'DISSABS, AGRICOLA, AQUASCI, BIOTECHNO, COMPENDEX, HEALSAFE,
LIFESCI, OCEAN, PASCAL, ADISCTI, ADISINSIGHT, ANTE, BIOENG, BIOSIS,
BIOTECHDS, CABA, CAPLUS, CEABA-VTB, CROPU, DDFB, DGENE, DRUGB, DRUGU,
EMBAL, EMBASE, ESBIODBASE, FEDRIP, GENBANK, IFIPAT, ...' ENTERED AT
09:38:59 ON 10 MAR 2006

L4 4222 DUP REM L3 (1190 DUPLICATES REMOVED)
L5 290 S L4 (S) (INTEGRIN OR COLLAGEN)
L6 39 S L5 (S) (ALPHA (A) 1 (A) BETA (A) 1)

=>

L6 ANSWER 21 OF 39 IFIPAT COPYRIGHT 2006 IFI on STN
 AN 10202710 IFIPAT;IFIUDB;IFICDB
 TITLE: METHOD FOR THE TREATMENT OF INFLAMMATORY DISORDERS;
 ADMINISTERING FUNCTION BLOCKING ANTIBODY OR A
 FRAGMENT ANTIBODY, CAPABLE OF BINDING AN EPIOTOPE OF
 VLA-1 TO PROVIDE A DECREASE IN ARTHRITIC SCORE OF
 ABOUT 65% OR GREATER WHEN COMPARED TO CONTROL
 ANTIBODY TREATED SUBJECT
 INVENTOR(S): De Fougerolles; Antonin, Brookline, MA, US
 Gotwals; Philip, West Roxbury, MA, US
 Kotelianski; Victor, Boston, MA, US
 Lobb; Roy, Westwood, MA, US
 PATENT ASSIGNEE(S): Unassigned
 PATENT ASSIGNEE PROBABLE: Biogen Idec MA Inc (Probable)
 AGENT: John T. Li BIOGEN, INC., 14 Cambridge Center,
 Cambridge, MA, 02142, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002146417	A1	20021010
APPLICATION INFORMATION:	US 2001-996738		20011130

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION OF:	WO 2000-US15004	20000601	UNKNOWN

	NUMBER	DATE
PRIORITY APPLN. INFO.:	US 1999-137038P	19990601 (Provisional)
	US 2000-185336P	20000229 (Provisional)
FAMILY INFORMATION:	US 2002146417	20021010
DOCUMENT TYPE:	Utility	
	Patent Application - First Publication	
FILE SEGMENT:	CHEMICAL APPLICATION	

NUMBER OF CLAIMS: 7 18 Figure(s).
 DESCRIPTION OF FIGURES:

FIG. 1. Collagen-binding integrins alpha

1 beta 1 and alpha 2 beta 1 on activated
 leukocytes. (A). Flow cytometric analysis of alpha 1 and
 alpha 2 beta 1 integrin expression on IL-2activated splenocytes (d
 11). Cells were labeled with either anti-alpha 1 mAb,
 anti-alpha 2 mAb, or non-binding control mAb (grey lines), and followed by
 FITC-anti-hamster immunoglobulin. (B) Effect of anti-alpha 1
 and anti-alpha 2 mAbs on leukocyte adhesion to collagen. 105 IL-2
 activated splenocytes were treated with indicated mAbs for 15 min before
 plating onto either type IV or type I collagen-coated wells for 1 h
 at 37 degrees C. Adhesion was calculated as illustrated in Example 1, and
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 were done in triplicate, and at least three independent experiments were
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FIG. 2. Effect of anti-integrin mAbs on the effector phase of
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 (anti-alpha 1), 29 (anti-alpha 2), 18 (antialpha
 1+anti-alpha 2), 45 (anti-alpha 4), 18 (anti-alpha 5), 20 (anti-alpha 6), and
 10 (anti-beta 1). The mAbs used were: Ha4/8 (control hamster Ig group 2),
 Ha31/8 (anti-alpha 1), Hal/ 29 (anti-alpha 2), PS/2
 (anti-alpha 4), 5H10-27 (anti-alpha 5), GoH3 (anti-alpha 6), and HM beta 1-1
 (anti-beta 1).

FIG. 3. Effect of anti-integrin mAbs on the effector phase of contact
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alpha 1), 44 (anti-alpha 2), 38 (anti-alpha
1 +antialpha 2), 36 (anti-alpha 4), 16 (anti-alpha 5), 26 (antialpha
4+anti-alpha 5), 24 (anti-alpha 6), and 22 (anti-beta 1). The hamster mAbs used were: Ha4/8 (control hamster Ig group 2), Ha31/8 (anti-alpha
1), Hal/29 (anti-alpha 2), HM beta 1-1 (anti-beta 1), 3E2 (anti-ICAM-1); the rat mAbs used were: R3595 and R35-38 (control rat IgG2a and rat IgG2b, respectively), PS/2 (anti-alpha 4), 5H10-27 (anti-alpha 5), GoH3 (antialpha 6).

FIG. 4. Contact hypersensitivity responses in alpha 1-deficient mice compared to wild-type mice. FITC-sensitized mice were
injected i.p. with indicated mAbs 4 h prior to FITC challenge. Ear thickness was measured at baseline and 24 h later, and results shown as % increase in ear thickness \pm -SEM as illustrated in Example 4. Groups of four to five mice per condition were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 5. Effect of anti-alpha 1 and anti-alpha 2 mAbs on croton oil-induced non-specific inflammation. Mice were
injected i.p. with indicated mAbs 4 h prior to ear painting with croton oil. Ear thickness was measured at baseline and 24 h later, and results shown as % increase in ear thickness \pm -SEM as illustrated in Example 5. Groups of four to five mice per condition were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 6. Effect of anti-alpha 1 and alpha 2 mAbs in
collagen mAb-induced arthritis. Mice were injected i.p. with anti-collagen mAbs at d 0, followed by LPS on day 3. Mice were
injected i.p. with indicated mAbs every 3rd day starting on d 0. Clinical arthritis was apparent 2-3 d following LPS injection and continued for several weeks. Each limb was evaluated on a 0 to 4 scale every 3rd day as illustrated in Example 6 and results are expressed as the mean arthritic score between d 9 and d 15 (\pm -SEM) of all four limbs. These data represent a summary of four experiments with each experiment consisting of groups of three to four mice per condition.

FIG. 7. Administration of anti-alpha 1 or anti-alpha 2 mAbs inhibits leukocyte infiltration into footpads during a DTH response. The experiment was performed as described in FIG. 2. Footpads were excised 20 h following antigen challenge and tissue sections stained with hematoxylin and eosin. Tissue sections are from footpads of either unchallenged mice (A) or SRBC-sensitized mice challenged with SRBC (B-H). Mice were treated 1 h prior to challenge with either PBS (B), control hamster Ig (C, G), anti-alpha
1 (D), anti-alpha 2 (E) or a combination of anti-alpha
1 and anti-alpha 2 mAbs (F,H). Magnification: x 100 (A-F), x 400 (G-H).

FIG. 8. alpha 1 beta 1 is expressed on infiltrating leukocytes in footpads during a DTH response. Immunohistochemical staining of infiltrating leukocytes from an untreated inflamed footpad 20 h after antigen challenge. (A). Serial sections stained directly with Alexa488-conjugated control mAb and anti-alpha 1 mAb. (B). Dual immunofluorescent staining with Alexa488conjugated anti-alpha
1 mAb and Phycoerythrin (PE)-conjugated cell lineage-specific mAbs. PE-conjugated mAbs utilized were specific for granulocytes/monocytes (anti-CD11b), neutrophils (anti-Ly6G/Gr-1), and T lymphocytes (anti-CD3). Magnification: x 400.

FIG. 9. Effect of anti-alpha 1 and alpha 2 mAbs in
collagen mAb-induced arthritis. (A). Preventative treatment of mice with either anti-alpha 1 or anti-alpha 2 mAb decreases arthritic score. Mice were treated with anti-collagen mAbs at d 0, followed by LPS on d 3. Arthritis was apparent by d 6 and continued for several weeks. Mice were treated with the indicated mAbs every 3rd day starting on d 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score between d 9 and d 15 (\pm -SEM) of all four limbs (maximum score of 16). Groups of 4 mice per condition were used; the average of 12 experiments is shown. (B). alpha 1-deficient mice have a reduced arthritic score comparable to anti-alpha 1 mAb-treated wild-type mice. Experimental details and scoring are as outlined above. Groups of 4 mice per condition were used; the average of 2 experiments is shown.

FIG. 10. Effect of anti-alpha 1 mAb treatment on the

immunopathology of arthritic joints. Anti-alpha 1 mAb treatment reduces leukocytic infiltration, adherence of cells to joint surfaces, and cartilage destruction as evidenced by proteoglycan loss. Hind limbs from normal mice (A-D) or arthritic mice (d 8) receiving either control hamster Ig (E-H) or anti-alpha 1 mAb treatment. (I-L). Limbs were photographed (A, E, I), excised, and tissue sections stained either with hematoxylin/eosin (B-C, F-G, J-K) or with toluidine blue to detect proteoglycan (D, H, L). Magnification: x 16 (B, F, J); x 160 (C, G, K); x 200 (D, H, L).

FIG. 11. Development of arthritis is delayed in the absence of lymphocytes and inhibition of arthritis by anti-alpha 1 mAb occurs in the absence of lymphocytes. Wild-type B6,129 or RAG-1deficient B6,129 mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated with the indicated mAbs every 3rd day starting on day 0. Each rd limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 12. Dose response of anti-alpha 1 mAb inhibition of arthritis. Wild-type Balb/c mice were treated with anticollagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with the indicated dose of either Ha4/8 (isotype control) or Ha31/8 (anti-alpha 1 mAbs every 3rd day starting on day 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 13. Therapeutic treatment with anti-alpha 1 mAb can decrease arthritic score. Wild-type Balb/c mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with mAbs (250 ug) or Ig fusion protein (200 ug) every 3d day starting on day 4. Mice received either mAb (Ha4/8 isotype control or Ha31/8 anti-alpha 1 mAb), Ig fusion protein (Isotype control Ig or TNF-R55-Ig) or a combination of both (250 ug Ha31/8 and 200 ug TNF-R55-Ig). Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 14. Location of the Epitope for the anti-alpha 1 domain Blocking mAbs. A. Amino acid sequence of the rat (top) and human (below) alpha 1-I domain. The residues that comprise the MIDAS (metal ion dependent adhesion site) motif are shown in bold. The human amino acids that replaced the corresponding rat residues (RAH) are shown below the rat sequence in the boxed region. For clarity, residue numbering in the text refers to this figure. B. Increasing concentrations of mAb AJH10 were bound to plates coated with 30 mu g/ml human (circles), rat (triangles) or R Delta H (squares) alpha 1-I domain. Data shown is representative of three experiments.

FIG. 15. Amino acid sequence of the human alpha 1-I domain.

DESCRIPTION OF FIGURES:

FIG. 16. Identification of a blocking mAb to the alpha 1-I domain. A. Increasing concentration of mAbs AEF3 (triangles) or AJH10 (circles) were bound to plates coated with 30 mu g/ml alpha 1-I domain. B. The alpha 1-I domain was treated with increasing concentrations of mAb AJH10 (diamonds) or mAb BGC5 (squares) and bound collagen IV (2 mu g/ml) coated plates. C. K562-alpha 1 cell were treated with increasing concentration of mAbs AEF3 (triangles) or AJH10 (circles) and bound to collagen IV (5 mu g/ml) coated plates. 45-50% of cells added to each well adhered to collagen IV. Data shown is representative of three independent experiments.

FIG. 17. Species Cross-reactivity of the blocking mAbs. A. Detergents lysates from (1) sheep vascular smooth muscle, (2) human leukemia K562-alpha 1 cells or (3) purified R Delta H GST-I domain; (4) Rat GST-alpha 1 I domain; and (5) human GSTalpha 1 I domain were separated by 10-20% SDS-PAGE under nonreducing conditions, and immunoblotted with function-blocking mAb AJH10. Molecular weight markers are shown on the left; nonreduced alpha 1 beta 1 integrin migrates at 180 kDa; GST-I domain migrates at 45 kDa. B. Rabbit vascular smooth muscle cells were incubated with either mAb AJH10 (bottom) or murine IgG control (top) and analyzed by fluorescence activated cell sorter (FACS).

FIG. 18. The alpha 1-I domain binds collagen. A. Increasing concentrations of the human alpha 1-I domain were bound to plates previously coated with 1 mu

g/ml collagen I (squares) or collagen IV (circles). Values shown have been corrected for background binding to BSA. B. 2 μ g/ml human alpha 1-I domain was mixed with increasing concentration of an anti-human alpha 1 integrin antibody 5E8D9 (squares) or an anti-human alpha 2 integrin antibody A2IIE10 (circles), and then bound to plates previously coated with 1 μ g/ml collagen IV. C. Plates were coated with 1 μ g/ml collagen IV or 3% BSA. alpha 1-I domain (2 μ g/ml) was subsequently bound to coated plates in the presence of 1 mM Mn²⁺, 1 mM Mg²⁺, or 5 mM EDTA. Data shown is representative of three independent experiments. !

AB A method for the treatment of inflammatory disorders is disclosed, particularly the treatment of arthritis. The method comprises the administration of a function blocking antibody which is capable of binding an epitope of VLA-1.

GI 18 Figure(s).

FIG. 1. Collagen-binding integrins alpha

1 beta 1 and alpha 2 beta 1 on activated leukocytes. (A). Flow cytometric analysis of alpha 1 and alpha 2 beta 1 integrin expression on IL-2 activated splenocytes (d 11). Cells were labeled with either anti-alpha 1 mAb, anti-alpha 2 mAb, or non-binding control mAb (grey lines), and followed by FITC-anti-hamster immunoglobulin. (B) Effect of anti-alpha 1 and anti-alpha 2 mAbs on leukocyte adhesion to collagen. 105 IL-2 activated splenocytes were treated with indicated mAbs for 15 min before plating onto either type IV or type I collagen-coated wells for 1 h at 37 degrees C. Adhesion was calculated as illustrated in Example 1, and expressed as %. . . done in triplicate, and at least three independent experiments were performed. One representative experiment is shown.

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L6 ANSWER 28 OF 39 USPATFULL on STN
 ACCESSION NUMBER: 2005:260856 USPATFULL
 TITLE: Method for the treatment of inflammatory disorders
 INVENTOR(S): Gotwals, Philip, Needham, MA, UNITED STATES
 DeFougerolles, Antonin, Brookline, MA, UNITED STATES
 Lobb, Roy, Westwood, MA, UNITED STATES
 Kotelianski, Victor, Boston, MA, UNITED STATES
 PATENT ASSIGNEE(S): BIOGEN IDEC, INC., Cambridge, MA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005226877	A1	20051013
APPLICATION INFO.:	US 2005-108581	A1	20050418 (11)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 2001-996738, filed on 30 Nov 2001, PENDING Continuation of Ser. No. WO 2000-US15004, filed on 1 Jun 2000, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-185336P	20000229 (60)
	US 1999-137038P	19990601 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	Kevin J. McGough, Esquire, Coleman, Sudol & Sapone, P.C., 714 Colorado Avenue, Bridgeport, CT, 06605-1601, US	
NUMBER OF CLAIMS:	20	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	19 Drawing Page(s)	
LINE COUNT:	1588	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for the treatment of inflammatory disorders is disclosed, particularly the treatment of arthritis. The method comprises the administration of a function blocking antibody which is capable of binding an epitope of VLA-1.

DETD Expression and functional blockade of .alpha.1.beta.1 and alpha2beta1 on activated leukocytes. Given the key role leukocytes play in inflammation, we decided to test whether anti-.alpha.1 and anti-alpha2 mAbs were capable of blocking leukocyte adhesion to collagens. In order to obtain leukocytes expressing high levels of both .alpha.1 and alpha2, murine T cells were stimulated in vitro with IL-2 for 7-12 d. These cells expressed high levels of both .alpha.1 and alpha2 (FIG. 1A), and bound well to both collagen type IV and type I-coated surfaces (FIG. 1B). Adhesion to type IV collagen was partially inhibited by anti-.alpha.1 mAb alone and was not inhibited by anti-alpha2 mAb alone. In contrast, adhesion to type I collagen was completely inhibited by anti-alpha2 mAb and anti-.alpha.1 mAb alone showed only partial inhibition. Both anti-beta1 mAb and the combination of anti-.alpha.1 and anti-alpha2 mAbs completely inhibited adhesion to types I and IV collagen. Having demonstrated that the .alpha.1.beta.1 and alpha2beta1 integrins are expressed on activated T cells and that anti-.alpha.1 and alpha2 mAbs are able to functionally block leukocyte adhesion to collagens, we used these mAbs to investigate the in vivo role of these integrins in animal models of inflammatory disorders.

L6 ANSWER 18 OF 39 IFIPAT COPYRIGHT 2006 IFI on STN
 AN 10574429 IFIPAT;IFIUDB;IFICDB
 TITLE: ANTIBODIES TO VLA-1; CRYSTAL STRUCTURE OF THE COMPLEX
 BETWEEN ONE SUCH ANTIBODY AND THE ALPHA 1-I DOMAIN OF
 VLA-1, AND TO THE USE OF THIS STRUCTURAL INFORMATION
 FOR COMPUTATIONAL DRUG DESIGN
 INVENTOR(S): Garber; Ellen A, Cambridge, MA, US
 Karpusas; Michael, Upper Darby, PA, US
 Lyne; Paul D, Arlington, MA, US
 Saldanha; Jose William B, Middlesex, GB
 PATENT ASSIGNEE(S): Unassigned
 PATENT ASSIGNEE PROBABLE: Biogen Idec MA Inc (Probable)
 AGENT: FISH & NEAVE, 1251 AVENUE OF THE AMERICAS, 50TH
 FLOOR, NEW YORK, NY, 10020-1105, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2004081651	A1	20040429
APPLICATION INFORMATION:	US 2003-474832		20031014
	WO 2002-US11521		20020412
			20031014 PCT 371 date
			20031014 PCT 102(e) date
FAMILY INFORMATION:	US 2004081651		20040429
DOCUMENT TYPE:	Utility		
	Patent Application - First Publication		
FILE SEGMENT:	CHEMICAL		
	APPLICATION		

NUMBER OF CLAIMS: 42 23 Figure(s).
 DESCRIPTION OF FIGURES:

FIG. 1. Collagen-binding integrins alpha
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FIG. 3. Effect of anti-integrin mAbs on the effector phase of contact
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 alpha 1), 44 (anti-alpha 2), 38 (anti-alpha
 1 +antialpha 2), 36 (anti-alpha 4), 16 (anti-alpha 5), 26 (antialpha
 4+anti-alpha 5), 24 (anti-alpha 6), and 22 (anti-beta 1). The hamster mAbs used
 were: Ha4/8 (control hamster Ig group 2), Ha31/8 (anti-alpha
 1), Ha1/29 (anti-alpha 2), HM beta 1-1 (anti-beta 1), 3E2
 (anti-ICAM-1); the rat mAbs used were: R3595 and R35-38 (control rat IgG2a and

rat IgG2b, respectively), PS/2 (anti-alpha 4), 5H10-27 (anti-alpha 5), G0H3 (anti-alpha 6).

FIG. 4. Contact hypersensitivity responses in alpha 1

-deficient mice compared to wild-type mice. FITC-sensitized mice were ***injected*** i.p. with indicated mAbs 4 h prior to FITC challenge. Ear thickness was measured at baseline and 24 h later, and results shown as % increase in ear thickness +-SEM as illustrated in Example 4. Groups of four to five mice per condition were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 5. Effect of anti-alpha 1 and anti-alpha 2 mAbs on

croton oil-induced non-specific inflammation. Mice were ***injected*** i.p. with indicated mAbs 4 h prior to ear painting with croton oil. Ear thickness was measured at baseline and 24 h later, and results shown as % increase in ear thickness +-SEM as illustrated in Example 5. Groups of four to five mice per condition were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 6. Effect of anti-alpha 1 and alpha 2 mAbs in

collagen mAb-induced arthritis. Mice were injected i.p. with anti-collagen mAbs at d 0, followed by LPS on day 3. Mice were ***injected*** i.p. with indicated mAbs every 3rd day starting on d 0.

Clinical arthritis was apparent 2-3 d following LPS injection and continued for several weeks. Each limb was evaluated on a 0 to 4 scale every 3rd day as illustrated in Example 6 and results are expressed as the mean arthritic score between d 9 and d 15 (+-SEM) of all four limbs. These data represent a summary of four experiments with each experiment consisting of groups of three to four mice per condition.

FIG. 7. Effect of anti-alpha 1 and alpha 2 mAbs in

collagen mAb-induced arthritis. A. Preventative treatment of mice with either anti-alpha 1 or anti-a2 mAb decreases arthritic score. Mice were treated with anti-collagen mAbs at d 0, followed by LPS on d 3. Arthritis was apparent by d 6 and continued for several weeks. Mice were treated with the indicated mAbs every 3rd day starting on d 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score between d 9 and d 15 (+-SEM) of all four limbs (maximum score of 16). Groups of 4 mice per condition were used; the average of 12 experiments is shown. B. alpha 1-deficient mice have a reduced arthritic score comparable to anti-alpha 1 mAb-treated wild-type mice. Experimental details and scoring are as outlined above. Groups of 4 mice per condition were used; the average of 2 experiments is shown.

FIG. 8. Development of arthritis is delayed in the absence of lymphocytes and inhibition of arthritis by anti-alpha 1 mAb occurs in the absence of lymphocytes. Wild-type B6,129 or RAG-1-deficient B6,129 mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated with the indicated mAbs every 3rd day starting on day 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 9. Dose response of anti-al mAb inhibition of arthritis. Wild-type Balb/c mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with the indicated dose of either Ha4/8 (isotype control) or Ha31/8 (anti-alpha 1) mAbs every 3rd day starting on day 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 10. Therapeutic treatment with anti-alpha 1 mAb can

decrease arthritic score. Wild-type Balb/c mice were treated with anti-***collagen*** mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with mAbs (250 mu g) or Ig fusion protein (200 mu g) every 3rd day starting on day 4. Mice received either mAb (Ha4/8 isotype control or Ha31/8 antialpha 1), Ig fusion protein (Isotype control Ig or TNF-R55-Ig) or a combination of both (250 ug Ha31/8 and 200 ug TNF-R55-Ig). Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 11. Location of the Epitope for the anti-alpha 1 I

domain Blocking nabs. A. Amino acid sequence of the rat (top; SEQ ID NO:63) and human (below; SEQ ID NO:64) alpha 1-I domain. The residues

that comprise the MIDAS (metal ion dependent adhesion site) motif are shown in bold. The human amino acids that replaced the corresponding rat residues (R Delta H) are shown below the rat sequence in the boxed region. For clarity, residue numbering in the text refers to this figure,, unless otherwise designated, e.g., as crystal numbering. B. Increasing concentrations of mAb AJH10 (ATCC No. PTA-3580; deposited under the Budapest Treaty with the American Type Culture Collection, Manassas, Va., USA on Aug. 2, 2001) were bound to plates coated with 30 μ g/ml human (circles), rat (triangles) or R Delta H (squares) alpha 1-I domain. Data shown is representative of three experiments.

FIG. 12. Amino acid sequence of the human alpha 1-I domain (SEQ ID NO:64).

FIG. 13. Identification of a blocking mAb to the alpha 1-I domain. A. Increasing concentration of mAbs AEF3 (triangles) or AJH10 (circles) were bound to plates coated with 30 μ g/ml alpha 1-I domain. B. The alpha 1-I domain was treated with increasing concentrations of mAb AJH10 (diamonds) or mAb BGC5 (squares) and bound ***collagen*** IV (2 μ g/ml) coated plates. C. K562-alpha ***1*** cell were treated with increasing concentration of mAbs AEF3(triangles) or AJH10 (circles) and bound to collagen IV (5 μ g/ml) coated plates. 45-50% of cells added to each well adhered to ***collagen*** IV. Data shown is representative of three independent experiments.

FIG. 14. Species Cross-reactivity of the blocking mAbs analyzed by fluorescence activated cell sorter (FACS). Rabbit vascular smooth muscle cells were incubated with either mAb AJH10 (bottom) or murine IgG control (top) and analyzed by fluorescence activated cell sorter (FACS).

FIG. 15. The alpha 1-I domain binds collagen. A. Increasing concentrations of the human alpha 1-I domain were bound to plates previously coated with 1 μ g/ml collagen I (squares) or collagen IV (circles). Values shown have been corrected for background binding to BSA. B. 2 μ g/ml human alpha 1-I domain was mixed with increasing concentration of an anti-human alpha ***1*** integrin antibody 5E8D9 (squares) or an anti-human alpha 2integrin antibody A2IIE10 (circles), and then bound to plates previously coated with 1 μ g/ml collagen IV. C. Plates were coated with 1 μ g/ml collagen IV or 3% BSA. alpha 1-I domain (2 μ g/ml) was subsequently bound to coated plates in the presence of 1 mM Mn²⁺, 1 mM Mg²⁺, or 5 mM EDTA. Data shown is representative of three independent experiments.

FIG. 16. Characterization of Humanized AQC2 Forms. mAQC2 (triangles), chAQC2 (circles), hAQC2 (inverted triangles) and hAQC2' (squares) were evaluated.

FIG. 17. Characterization of Humanized AQC2 Forms by FACS.

FIG. 18. Characterization of Humanized AQC2 Forms by FACS.

DESCRIPTION OF FIGURES:

FIG. 19. Atomic structure coordinates for the alpha 1-I domain/ Fab complex, as derived by X-ray crystallography from crystals of that complex in Protein Data Bank (PDB) format. The coordinates of the two complexes in the asymmetric unit are listed as follows.

FIG. 20. I domain-Fab complex. A. Ribbon diagram of the I domainFab complex. The I domain is colored green and the antibody heavy and light chain yellow and blue, respectively. The Mn²⁺ ion is the white colored sphere. B. Close-up of the MIDAS (Metal-Ion-Dependent-Adhesion-Site) site showing the coordination of the metal ion (white sphere) by Asp101 (crystal numbering). The protein backbones are shown as ribbons and the side chains in the ball-and-stick representation. The blue cylinders represent interactions between the metal ion and protein atoms. The thin lines represent H-bonds. FIG. 20 was made with the software program RIBBONS (Carson, 1991, J. Appl. Cryst. 24:958-961).

FIG. 21. A diagram of a system used to carry out the instructions encoded by the storage medium of FIGS. 22 and 23.

FIG. 22. A cross section of a magnetic storage medium.

FIG. 23. A cross section of an optically-readable data storage medium.!

AB Antibodies that specifically bind to VLA-1 integrin and methods of using these antibodies to treat immunological disorders in a subject. Also included are crystal structures of complexes formed by VLA-1 antibodies and their ligands, and VLA-1 antagonists and agonists identified by using the structure coordinates of these structures.

GI 23 Figure(s).

FIG. 1. Collagen-binding integrins alpha

1 beta 1 and alpha 2 beta 1 on activated leukocytes. (A). Flow cytometric analysis of alpha 1 and alpha 2 beta 1 integrin expression on IL-2activated splenocytes (d 11). Cells were labeled with either anti-alpha 1 mAb, anti-alpha 2 mAb, or non-binding control mAb (grey lines), and followed by FITC-anti-hamster immunoglobulin. (B) Effect of anti-alpha 1 and anti-alpha 2 mAbs on leukocyte adhesion to collagen. 105 IL-2 activated splenocytes were treated with indicated mAbs for 15 min before plating onto either type IV or type I collagen-coated wells for 1 h at 37 degrees C. Adhesion was calculated as illustrated in Example 1, and expressed as %. . . were done in triplicate, and at least three independent experiments were performed. One representative experiment is shown.

FIG. 2. Effect of anti-integrin mAbs on the effector phase of delayed-type hypersensitivity. SRBC-sensitized mice were injected i.p. with the indicated mAbs 1 h prior to SRBC challenge. Footpad thickness was measured 20 h after antigen challenge, . . . illustrated in Example 2. These data represent a summary of eight experiments with n=79 (PBS), 68 (control hamster Ig), 68 (anti-alpha 1), 29 (anti-alpha 2), 18 (anti-alpha 1+anti-alpha 2), 45 (anti-alpha 4), 18 (anti-alpha 5), 20 (anti-alpha 6), and 10 (anti-beta 1). The mAbs used were: Ha4/8 (control hamster Ig group 2), Ha31/8 (anti-alpha 1), Ha1/29 (anti-alpha 2), PS/2 (anti-alpha 4), 5H10-27 (anti-alpha 5), GoH3 (anti-alpha 6), and HM beta 1-1 (anti-beta 1).

FIG. 3. Effect of anti-integrin mAbs on the effector phase of contact hypersensitivity. FITC-sensitized mice were injected i.p. with the indicated mAbs 4 h prior to FITC challenge. Ear thickness was measured at baseline and 24. . . Example 3. These data represent a summary of nine experiments with n=74 (PBS), 60 (control hamster Ig), 26 (anti-ICAM-1), 44 (anti-alpha 1), 44 (anti-alpha 2), 38 (anti-alpha 1+anti-alpha 2), 36 (anti-alpha 4), 16 (anti-alpha 5), 26 (anti-alpha 4+anti-alpha 5), 24 (anti-alpha 6), and 22 (anti-beta 1). The hamster mAbs used were: Ha4/8 (control hamster Ig group 2), Ha31/8 (anti-alpha 1), Ha1/29 (anti-alpha 2), HM beta 1-1 (anti-beta 1), 3E2 (anti-ICAM-1); the rat mAbs used were: R3595 and R35-38 (control rat IgG2a and rat IgG2b, respectively), PS/2 (anti-alpha 4), 5H10-27 (anti-alpha 5), GoH3 (anti-alpha 6).

FIG. 4. Contact hypersensitivity responses in alpha 1-deficient mice compared to wild-type mice. FITC-sensitized mice were injected i.p. with indicated mAbs 4 h prior to FITC challenge. Ear thickness was measured at baseline and 24 h later, . . . were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 5. Effect of anti-alpha 1 and anti-alpha 2 mAbs on croton oil-induced non-specific inflammation. Mice were injected i.p. with indicated mAbs 4 h prior to ear painting with croton oil. Ear thickness was measured at baseline and . . . were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 6. Effect of anti-alpha 1 and alpha 2 mAbs in collagen mAb-induced arthritis. Mice were injected i.p. with anti-collagen mAbs at d 0, followed by LPS on day 3. Mice were injected i.p. with indicated mAbs every 3rd day starting on d 0. Clinical arthritis was apparent 2-3 d following LPS injection and continued for several weeks. Each limb was evaluated on a 0 to 4 scale every 3rd day as illustrated. . . summary of four experiments with each experiment consisting of groups of three to four mice per condition.

FIG. 7. Effect of anti-alpha 1 and alpha 2 mAbs in collagen mAb-induced arthritis. A. Preventative treatment of mice with either anti-alpha 1 or anti-alpha 2 mAb decreases arthritic score. Mice were treated with anti-collagen mAbs at d 0, followed by LPS on d 3. Arthritis was apparent by d 6 and continued for several. . . were used; the average of 12 experiments is shown. B. alpha 1-deficient mice have a reduced arthritic score comparable to anti-alpha 1 mAb-treated wild-type mice. Experimental details and scoring are as outlined above. Groups of 4 mice per condition were used; the. . . 2 experiments is shown.

FIG. 8. Development of arthritis is delayed in the absence of lymphocytes

and inhibition of arthritis by anti-alpha 1 mAb occurs in the absence of lymphocytes. Wild-type B6,129 or RAG-1deficient B6,129 mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several. . . mice per condition were used.

FIG. 9. Dose response of anti-al mAb inhibition of arthritis. Wild-type Balb/c mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with the indicated dose of either Ha4/8 (isotype control) or Ha31/8 (anti-alpha 1) mAbs every 3rd day starting on day 0. Each limb was evaluated and scored on a 0 to 4 scale. . . arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 10. Therapeutic treatment with anti-alpha 1 mAb can decrease arthritic score. Wild-type Balb/c mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several. . . limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 11. Location of the Epitope for the anti-alpha 1 I domain Blocking nabs. A. Amino acid sequence of the rat (top; SEQ ID NO:63) and human (below; SEQ ID NO:64) alpha 1-I domain. The residues that comprise the MIDAS (metal ion dependent adhesion site) motif are shown in bold. The human amino. . . 2, 2001) were bound to plates coated with 30 mu g/ml human (circles), rat (triangles) or R Delta H (squares) alpha 1-I domain. Data shown is representative of three experiments.

FIG. 12. Amino acid sequence of the human alpha 1-I domain (SEQ ID NO:64).

FIG. 13. Identification of a blocking mab to the alpha 1 -I domain. A. Increasing concentration of mAbs AEF3 (triangles) or AJH10 (circles) were bound to plates coated with 30 mu g/ml alpha 1-I domain. B. The alpha 1-I domain was treated with increasing concentrations of mAb AJH10 (diamonds) or mAb BGC5 (squares) and bound collagen IV (2 mu g/ml) coated plates. C. K562-alpha 1 cell were treated with increasing concentration of mAbs AEF3(triangles) or AJH10 (circles) and bound to collagen IV (5 mu g/ml) coated plates. 45-50% of cells added to each well adhered to collagen IV. Data shown is representative of three independent experiments.

FIG. 14. Species Cross-reactivity of the blocking mAbs analyzed by fluorescence activated. . . with either mAb AJH10 (bottom) or murine IgG control (top) and analyzed by fluorescence activated cell sorter (FACS).

FIG. 15. The alpha 1-I domain binds collagen . A. Increasing concentrations of the human alpha 1-I domain were bound to plates previously coated with 1 mu g/ml collagen I (squares) or collagen IV (circles). Values shown have been corrected for background binding to BSA. B. 2 mu g/ml human alpha 1-I domain was mixed with increasing concentration of an anti-human alpha 1 integrin antibody 5E8D9 (squares) or an anti-human alpha 2integrin antibody A2IIE10 (circles), and then bound to plates previously coated with 1 mu g/ml collagen IV. C. Plates were coated with 1 mu g/ml collagen IV or 3% BSA. alpha 1-I domain (2 mu g/ml) was subsequently bound to coated plates plates in the presence of 1 mM Mn2+, 1 mM. . .

L6 ANSWER 19 OF 39 IFIPAT COPYRIGHT 2006 IFI on STN

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TITLE: METHODS AND COMPOSITIONS RELATING TO ISOLEUCINE BOROPROLINE COMPOUNDS; ABNORMAL CELL PROLIFERATION; INFECTIOUS DISEASES; INJECTION, ENTERICALLY COATED ORAL ADMINISTRATION

INVENTOR(S): Adams; Sharlene, Waltham, MA, US
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Atlantic Avenue, Boston, MA, 02210, US

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PATENT INFORMATION:	US 2004077601	A1	20040422
APPLICATION INFORMATION:	US 2003-616694		20030709

	NUMBER	DATE
PRIORITY APPLN. INFO.:	US 2002-394856P	20020709 (Provisional)
	US 2002-414978P	20021001 (Provisional)
	US 2003-466435P	20030428 (Provisional)
FAMILY INFORMATION:	US 2004077601	20040422
DOCUMENT TYPE:	Utility	
	Patent Application - First Publication	
FILE SEGMENT:	CHEMICAL	
	APPLICATION	

PARENT CASE DATA:

This application claims priority under 35 U.S.C. section 119(e) to U.S. Provisional Application Serial No. 60/394,856, entitled "BOROPROLINE COMPOUND COMBINATION THERAPY", filed on Jul. 9, 2002; U.S. Provisional Application Serial No. 60/414,978, entitled "BOROPROLINE COMPOUND COMBINATION THERAPY", filed on Oct. 1, 2002; and U.S. Provisional Application Serial No. 60/ 466,435, entitled "BOROPROLINE COMPOUND COMBINATION THERAPY", filed on Apr. 28, 2003, each of which is herein incorporated by reference in its entirety.

NUMBER OF CLAIMS: 484 2 Figure(s).
DESCRIPTION OF FIGURES:

FIG. 1. Comparison of the ability of Ile-boroPro to stimulate chemokine production in vivo when administered orally or by subcutaneous injection. Ile-boroPro was administered to mice by oral gavages (open symbols) or by subcutaneous injection (closed symbols) at the doses indicated on the abscissa. Two hours after administration, serum samples were obtained and assayed for DPP-IV activity (Delta / up-triangle-filled), using the fluorogenic substrate Ala-Pro-7-amino-4trifluoromethyl coumarin, and the chemokine KC (large-circle / circle-solid) by ELISA.

FIG. 2. Comparison of the anti-tumor activity of Ile-boroPro administered orally or by subcutaneous injection in the WEHI 164 mouse tumor model. Mice were inoculated subcutaneously with 4 x 10⁶ tumor cells and administered Ile-boroPro twice daily from day 2 to day 19 after tumor inoculation, either by oral gavage (open bars) or subcutaneous injection (solid bars). Control mice received saline (hatched bar). The data represent mean tumor volumes+SEM (n=10) were recorded on day 20. Treatment with all 3 doses of Ile-boroPro resulted in significant reduction in tumor sizes (oral administration: P less-than 0.05; subcutaneous administration: P less-than 0.00005).

AB A method for treating subjects with, inter alia, abnormal cell proliferation or infectious disease. Compositions containing Ile-boroPro compounds are also provided. The invention embraces the use of these compounds alone or in combination with other therapeutic agents.

ACLM 256. The method of claim 192, 194, 198 or 203, wherein the cancer antigen is a cancer testis (CT) antigen.

ACCESSION NUMBER: 2000-04343 BIOTECHDS

TITLE: Novel methods for treating kidney disorders using an
alpha-1-beta-1 integrin receptor-inhibitor and
TGF-beta-1-inhibitor;
integrin receptor-inhibitor and transforming growth
factor-inhibitor for use in therapy

AUTHOR: Cosgrove D

PATENT ASSIGNEE: Boys-Town-Nat.Res.Hosp.Omaha

LOCATION: Omaha, NE, USA.

PATENT INFO: WO 9961040 2 Dec 1999

APPLICATION INFO: WO 1999-US11073 19 May 1999

PRIORITY INFO: US 1998-150485 9 Sep 1998; US 1998-86587 22 May 1998

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2000-116327 [10]

AB Using alpha-1,beta-1

integrin receptor-inhibitor (I) and transforming growth
factor-beta-1-inhibitor (II) for therapy of kidney disorders is
claimed. (I) is given to a patient to limit a kidney disorder.
Also claimed are: delaying onset of and/or slowing progression of Alport
Syndrome (AS) in a patient by administering an agent that
inhibits signal transduction via an alpha-1,
beta-1 integrin receptor (A1B1IR) of a
kidney cell or by blocking an A1B1IR binding site on the surface
of a patient's kidney cell; delaying onset of and/or slowing
progression of kidney disease in insulin-dependent diabetes
mellitus (IDDM) in a patient by administering an agent that
inhibits signal transduction via an A1B1IR of a kidney cell;
delaying onset of and/or slowing progression of a kidney
disease in IDDM in a patient by blocking an A1B1IR binding site on the
surface of a patient's kidney cell; limiting kidney
fibrosis in a patient; a mouse model for kidney disease, where
the mouse does not express normal collagen type 4 in glomerular
basement membrane and does not express A1B1IR; drug screening; and
limiting matrix accumulation in AS patients. (93pp)

AB Using alpha-1,beta-1

integrin receptor-inhibitor (I) and transforming growth
factor-beta-1-inhibitor (II) for therapy of kidney disorders is
claimed. (I) is given to a patient to limit a kidney disorder.
Also claimed are: delaying onset of and/or slowing progression of Alport
Syndrome (AS) in a patient by administering an agent that
inhibits signal transduction via an alpha-1,
beta-1 integrin receptor (A1B1IR) of a
kidney cell or by blocking an A1B1IR binding site on the surface
of a patient's kidney cell; delaying onset of and/or slowing
progression of kidney disease in insulin-dependent diabetes
mellitus (IDDM) in a patient by administering an agent that
inhibits signal transduction via an A1B1IR of a kidney cell;
delaying onset of and/or slowing progression of a kidney
disease in IDDM in a patient by blocking an A1B1IR binding site on the
surface of a patient's kidney cell; limiting kidney
fibrosis in a patient; a mouse model for kidney disease, where
the mouse does not express normal collagen type 4 in glomerular
basement membrane and does not express A1B1IR; drug screening; and
limiting matrix accumulation in AS patients... .

10/09/99 573
492,325

ACCESSION NUMBER: 2003-07191 BIOTECHDS

TITLE: New anti-VLA-1 antibodies are useful for preventing or treating VLA-1-mediated immunological or inflammatory disorders, e.g. psoriasis, eczema, burns, dermatitis, and abnormal proliferation of hair follicle cells or fibrosis; vector-mediated recombinant protein gene transfer and expression in hybridoma cell culture for use in disease prevention and therapy

AUTHOR: LYNE P D; GARBER E A; SALDANHA J W; KARPUSAS M

PATENT ASSIGNEE: BIOGEN INC

PATENT INFO: WO 2002083854 24 Oct 2002

APPLICATION INFO: WO 2002-US11521 12 Apr 2002

PRIORITY INFO: US 2001-303689 6 Jul 2001; US 2001-283794 13 Apr 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-093009 [08]

AB DERWENT ABSTRACT:

NOVELTY - Anti-VLA-1 antibody (Ab) where the light chain complementarity determining regions (CDRs) are defined by amino acid residues 24-33, 49-55, and 88-96 of a fully defined sequence of 106 amino acids (S1) given in the specification, and where the heavy chain CDRs are defined by amino acid residues 31-35, 50-65, and 98-107 of a fully defined sequence of 118 amino acids (S2) given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a composition comprising (Ab) and a carrier; (2) an isolated nucleic acid comprising a coding sequence for: (a) the sequence of S1 or S2; (b) the light chain or heavy chain of an antibody produced by hybridoma mAQC2 (ATCC accession number PTA3273); (c) light chain or heavy chain of an antibody produced by cell line hAQC2 (ATCC accession number PTA3275); (d) the heavy chain of an antibody produced by cell line haAQC2 (ATCC accession number PTA3274) or cell line hsAQC2 (ATCC accession number PTA3356); (e) residues 1-106 of a fully defined sequence of 213 amino acids (S3) given in the specification; or (f) residues 1-118 of a fully defined sequence of 447 amino acids (S4) given in the specification; (3) treating a subject with an immunological disorder mediated by VLA-1 comprising administering to the subject the composition of (1); (4) determining the level of VLA-1 in a tissue comprising contacting the tissue with (Ab), and detecting the binding of the antibody to the tissue; (5) cells of: (a) hybridoma mAQC2 (ATCC accession number PTA3273); or (b) cell line hAQC2 (ATCC accession number PTA3275), haAQC2 (ATCC accession number PTA3274), or cell line hsAQC2 (ATCC accession number PTA3356); (6) identifying an inhibitor of an I domain of an integrin; (7) an inhibitor of I domain of integrin identified; and (8) computers for producing a three-dimensional (3-D) representation.

BIOTECHNOLOGY - Preferred Antibody: The antibody comprises: (a) a light chain variable domain sequence of S1 and a heavy chain variable domain sequence of S2; (b) the same heavy and light chain polypeptide sequences as an antibody produced by hybridoma mAQC2 (ATCC accession number PTA3273), cell line hAQC2 (ATCC accession number PTA3275), cell line hsAQC2 (ATCC accession number PTA3356), or cell line haAQC2 (ATCC accession number PTA3274); (c) at least one of the following residues in its light chain: Q1, L4, P46, W47, or Y71, or at least one of the following residues in its heavy chain: D1, V12, S28, F29, A49, T93, or R94 (Kabat numbering convention); (d) a light chain variable domain sequence defined by amino acid residues 1-106 of S3 and a heavy chain variable domain sequence defined by amino acid residues 1-118 of S4; (e) mutations L234A and L235A (EU numbering system) in its heavy chain as compared with an unmodified antibody; or (f) mutation N297Q in its heavy chain (EU numbering system). The antibody is preferably a humanized antibody. The heavy chain is mutated at one or more of amino acid residues selected from the group of residues 234, 235, 236, 237, 297, 318, 320 or 322 (EU numbering system), which causes an alteration in an effector function while retaining binding to VLA-1 as compared with an unmodified antibody. The antibody is mutated at an amino acid residue that is a glycosylation site to eliminate the glycosylation site.

Preferred Method: Identifying an inhibitor of an I domain of an integrin comprises: (a) using the structure coordinates of: (i) hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification, or +/- a root mean square deviation from the backbone atoms of the hAQC2 amino acids of not more than 1.10 Angstrom; (ii) I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification; or (iii) at least three of I domain amino acids comprising residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification, or +/- a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.30 Angstrom; to generate a 3-D structure of a binding site; (b) employing the 3-D structure to design or select a potential antagonist; (c) synthesizing the potential antagonist; and (d) contacting the potential antagonist with hAQC2 or I domain to determine the ability of the potential antagonist to interact with hAQC2 or I domain, where the ability of the potential antagonist to interact with hAQC2 indicates that the potential antagonist is an inhibitor of the I domain.

Preferred Computer: The computer for producing a 3-D representation of: (a) a molecular complex comprising the set of structure coordinates of a complex of a chimeric I domain of an alphabeta1 integrin RDELTAH and a humanized antibody hAQC2, defined in the specification, or a homologue of the molecular complex having a root mean square deviation from the backbone atoms of the amino acids of not more than 0.65 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises at least a portion of the structure coordinates of the complex, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D representation; (b) a molecule or molecular complex comprising a first binding site defined by structure coordinates of hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification, or a homologue of the molecule or molecular complex, which comprises a second binding site having a root mean square deviation from the backbone atoms of the hAQC2 amino acids of not more than 1.10 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D representation; (c) a first binding site defined by structure coordinates of hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification, or a second binding site having a root mean square deviation from the backbone atoms of the hAQC2 amino acids of not more than 1.10 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable

data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D; (d) a molecule or molecular complex comprising a first binding site defined by structure coordinates of I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification, or a homologue of the molecule or molecular complex, which comprises a second binding site having a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.92 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D; (e) a first binding site defined by structure coordinates of I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification, or a second binding site of a homologue having a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.92 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D; (f) a molecule or molecular complex comprising a first binding site defined by structure coordinates of I domain amino acids comprising at least three of residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification, or a homologue of the molecule or molecular complex, which comprises a second binding site having a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.30 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of I domain amino acids comprising at least three of residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D; or (g) a first binding site defined by structure coordinates of I domain amino acids comprising at least three of residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification, or a second binding site having a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.30 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of I domain amino acids comprising at least three of residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D.

ACTIVITY - Antiallergic; Antiinflammatory; Immunosuppressive; Antianemic; Antiarteriosclerotic; Antiasthmatic; Antithyroid; Vasotropic; Vulnerary; Cytostatic; Antipsoriatic; Dermatological; Antidiabetic; Antibacterial; Nephrotropic; Ophthalmological; Antimigraine; Antipyretic;

Antirheumatic; Antiarthritic; Neuroprotective; Osteopathic. Arthritis was induced in mice by injection of a cocktail of anti-collagen type II monoclonal antibodies (mAbs) on day 0, followed by LPS administration on day 3. Mice were then treated with either anti-alpha mAb or a soluble TNF receptor Ig fusion protein starting on day 4. Progression of arthritis was completely blocked in mice receiving anti-alpha mAb starting at day 4, when compared to mice receiving control hamster mAb starting at day 4. The degree of inhibition seen with therapeutic administration of anti-alpha mAb was complete and was equal to that seen with preventive treatment of anti-alpha mAb (started at day 0). In comparison, treatment with TNF receptor Ig fusion protein from day 4 onwards resulted in only a 60-70% inhibition in arthritic score when compared to control Ig fusion protein. Combined treatment of anti-alpha mAb and TNF receptor Ig fusion together was effective at completely inhibiting arthritic score.

MECHANISM OF ACTION - Integrin Antagonist Alpha

1 Beta 1.

USE - The anti-VLA-1 antibodies are useful for preventing or treating VLA-1-mediated immunological or inflammatory disorders, such as skin related conditions (e.g. psoriasis, eczema, burns, dermatitis, and abnormal proliferation of hair follicle cells), fibrosis (e.g. kidney or lung fibrosis), allergic rhinitis, respiratory distress syndrome, asthma, bronchitis, tendonitis, bursitis, fever, migraine headaches, gastrointestinal conditions (e.g. inflammatory bowel disease, Crohn's disease, gastritis, irritable bowel syndrome, colitis and colorectal cancer), vascular diseases (e.g. atherosclerosis), periarteritis nodosa, thyroiditis, aplastic anemia, Hodgkin's disease, rheumatic fever, osteoarthritis, autoimmune diseases (e.g. type I diabetes, myasthenia gravis, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis), sarcoidosis, nephrotic syndrome, renal failure, Behcet's syndrome, polymyositis, gingivitis, hypersensitivity (e.g. delayed type hypersensitivity or immediate hypersensitivity), graft and transplant rejections, graft versus host disease, conjunctivitis, swelling occurring after injury, myocardial ischemia, or endotoxin shock syndrome. The structural information can be used for computational drug design.

ADMINISTRATION - Dosage is about 0.001-100 (preferably 0.1-50) mg/kg/day. Administration may be oral, topical, intravenous, subcutaneous, intraperitoneal, intramuscular, intramedullary, intraarterial, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intraspinal, intracranial, locally at sites of inflammation or tumor growth, by inhalation, or by implantation of an infusion pump or a biocompatible sustained release implant into the subject.

EXAMPLE - Robertsonian mice were immunized intraperitoneally (i.p.) with 25 mug of purified human alphabeta1 emulsified with complete Freund's adjuvant. The mouse with the highest anti-alpha-1 domain titer was boosted i.p. with 100 mug of alphabeta1 three days prior to fusion, and intravenously with 50 mug of alphabeta1 one day prior to fusion. Spleen cells were fused with FL653 myeloma cells at a 1:6 ratio and were plated at 100000 and 33000 per well into 96-well tissue culture plates. Supernatants were assessed for binding to the alphabeta1 integrin by single color FACS. Supernatants from the resulting hybridomas were screened for binding to the alpha-1 domain. Selected supernatants were tested for their ability to inhibit K562-alpha1 dependent adhesion to Collagen IV. K562-alpha1 cells were labeled with 2mM 2'7' (bis-2-carboxyethyl-5 and -6) carboxyfluorescein penta acetoxymethylester (BCECF; Molecular Probes) in DMEM containing 0.25% BSA at 37degreesC for 30 minutes. Labeled cells were washed with binding buffer and re-suspended in binding buffer plus 5 mM MgCl2 at a final concentration of 1 x 10⁶ cells/ml. 50 mul of supernatant was incubated with an equal volume of 2 x 10⁵ K562-alpha1 cells in wells of a 96-well plate. The plate was then centrifuged and the supernatants removed. Cells were re-suspended in binding buffer and transferred to wells of a collagen-coated plate and incubated for 1 hour at 37degreesC. Following incubation, the non-adherent cells were removed by washing three times with binding buffer. Nineteen hybridomas were initially identified, the supernatants

of which bound to human leukemia K562 cells expressing the alphabeta1 integrin (K562-alpha1) and to the alpha1-I domain. The immunoglobulins were purified from each of these hybridomas and tested for the ability to block either K562-alpha1 or alpha1-I domain binding to collagen IV. The mAbs produced by clones AEF3, BGC5, AQC2 and AJH10 bind the alpha1-I domain, only mAbs AJH10 and AQC2 inhibit alpha1-I domain-dependent, or K562-alpha1 adhesion to collagen IV. (248 pages)

AB DERWENT ABSTRACT:

NOVELTY - Anti-VLA-1 antibody (Ab) where the light chain complementarity determining regions (CDRs) are defined by amino acid residues 24-33, 49-55, and 88-96. . . . sequence of 447 amino acids (S4) given in the specification; (3) treating a subject with an immunological disorder mediated by VLA-1 comprising administering to the subject the composition of (1); (4) determining the level of VLA-1 in a tissue comprising contacting the tissue with (Ab), and detecting the binding of the antibody to the tissue; (5). . . . number PTA3274), or cell line hsAQC2 (ATCC accession number PTA3356); (6) identifying an inhibitor of an I domain of an integrin; (7) an inhibitor of I domain of integrin identified; and (8) computers for producing a three-dimensional (3-D) representation.

BIOTECHNOLOGY - Preferred Antibody: The antibody comprises: (a) a light. . . . 297, 318, 320 or 322 (EU numbering system), which causes an alteration in an effector function while retaining binding to VLA-1 as compared with an unmodified antibody. The antibody is mutated at an amino acid residue that is a glycosylation site to eliminate the glycosylation site. Preferred Method: Identifying an inhibitor of an I domain of an integrin comprises: (a) using the structure coordinates of: (i) hAQC2 amino acids comprising at least 7 of light chain residues Asn30,. . . . a molecular complex comprising the set of structure coordinates of a complex of a chimeric I domain of an alphabeta1 integrin RDELTAH and a humanized antibody hAQC2, defined in the specification, or a homologue of the molecular complex having a root. . . . Vulnerary; Cytostatic; Antipsoriatic; Dermatological; Antidiabetic; Antibacterial; Nephrotropic; Ophthalmological; Antimigraine; Antipyretic; Antirheumatic; Antiarthritic; Neuroprotective; Osteopathic. Arthritis was induced in mice by injection of a cocktail of anti-collagen type II monoclonal antibodies (mAbs) on day 0, followed by LPS administration on day 3. Mice were then treated with either anti-alpha1 mAb or a soluble TNF receptor Ig fusion protein starting on day 4. Progression of arthritis was completely blocked in mice receiving anti-alpha1 mAb starting at day 4, when compared to mice receiving control hamster mAb starting at day 4. The degree of inhibition seen with therapeutic administration of anti-alpha1 mAb was complete and was equal to that seen with preventive treatment of anti-alpha1 mAb (started at day 0). In comparison, treatment with TNF receptor Ig fusion protein from day 4 onwards resulted in only a 60-70% inhibition in arthritic score when compared to control Ig fusion protein. Combined treatment of anti-alpha1 mAb and TNF receptor Ig fusion together was effective at completely inhibiting arthritic score.

MECHANISM OF ACTION - Integrin Antagonist Alpha 1 Beta 1.

USE - The anti-VLA-1 antibodies are useful for preventing or treating VLA-1-mediated immunological or inflammatory disorders, such as skin related conditions (e.g. psoriasis, eczema, burns, dermatitis, and abnormal proliferation of hair follicle cells), fibrosis (e.g. kidney or lung fibrosis), allergic rhinitis, respiratory distress syndrome, asthma, bronchitis, tendonitis, bursitis, fever, migraine headaches, gastrointestinal conditions (e.g. inflammatory bowel. . . . be oral, topical, intravenous, subcutaneous, intraperitoneal, intramuscular, intramedullary, intraarterial, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intraspinal, intracranial, locally at sites of inflammation or tumor growth, by inhalation, or by implantation of an infusion pump or a biocompatible sustained release implant into the. . . . immunized intraperitoneally (i.p.) with 25 mug of purified

human $\alpha\beta$ 1 emulsified with complete Freund's adjuvant. The mouse with the highest anti- α 1-I domain titer was boosted i.p. with 100 μ g of $\alpha\beta$ 1 three days prior to fusion, and intravenously with 50 μ g. . . plated at 100000 and 33000 per well into 96-well tissue culture plates. Supernatants were assessed for binding to the $\alpha\beta$ 1 integrin by single color FACS. Supernatants from the resulting hybridomas were screened for binding to the α 1-I domain. Selected supernatants were tested for their ability to inhibit K562- α 1 dependent adhesion to Collagen IV. K562- α 1 cells were labeled with 2mM 2'7' (bis-2-carboxyethyl-5 and -6) carboxyfluorescein penta acetoxymethylester (BCECF; Molecular Probes) in DMEM containing 0.25% BSA. . . concentration of 1×10^6 cells/ml. 50 μ l of supernatant was incubated with an equal volume of 2×10^5 K562- α 1 cells in wells of a 96-well plate. The plate was then centrifuged and the supernatants removed. Cells were re-suspended in binding buffer and transferred to wells of a collagen-coated plate and incubated for 1 hour at 37degreesC. Following incubation, the non-adherent cells were removed by washing three times with. . . binding buffer. Nineteen hybridomas were initially identified, the supernatants of which bound to human leukemia K562 cells expressing the $\alpha\beta$ 1 integrin (K562- α 1) and to the α 1-I domain. The immunoglobulins were purified from each of these hybridomas and tested for the ability to block either K562- α 1 or α 1-I domain binding to collagen IV. The mAbs produced by clones AEF3, BGC5, AQC2 and AJH10 bind the α 1-I domain, only mAbs AJH10 and AQC2 inhibit α 1-I domain-dependent, or K562- α 1 adhesion to collagen IV. (248 pages)

L6 ANSWER 4 OF 39 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
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 TITLE: Glomerular extracellular matrix components and integrins
 AUTHOR: Sterk L.M.Th.; De Melker A.A.; Kramer D.; Kuikman I.; Chand A.; Claessen N.; Weening J.J.; Sonnenberg A.
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AB It has become apparent that extracellular matrix components and their cellular receptors, the integrins, are important regulators of glomerular development and function. In this rapidly evolving field we studied the production of extracellular matrix components and integrins by rat glomerular visceral epithelial and mesangial cells, using molecular probes and antibodies that have recently become available. Special attention was paid to laminin isoforms and to splice variants of the integrin subunits $\alpha 3$ and $\alpha 6$. Results were compared to the in vivo expression in human fetal, newborn and adult kidneys. The mesangial cells were found to produce laminin-1, nidogen and two as yet unidentified laminin isoforms with putative α chains of about 395 (αx) and of 375 kDa (αy), tentatively described before as bovine kidney laminin. Furthermore, they expressed the integrins .alpha.1.beta.1, .alpha.2.beta.1, .alpha.3A.beta.1, .alpha.5.beta.1, .alpha.v β 3, $\alpha v\beta 5$, and small amounts of $\alpha 6A\beta 1$ and $\alpha 6B\beta 1$. The glomerular visceral epithelial cells produced the two new laminin isoforms mentioned above, laminin-5, but no laminin-1 or nidogen. The integrins $\alpha 2$.beta.1, .alpha.3A.beta.1, .alpha.6A $\beta 4$, $\alpha 6B\beta 4$ and the integrin subunit αv were found to be expressed. We show that during nephrogenesis, the laminin .alpha.1 chain disappears and is replaced by another α chain, possibly one of the two as yet unidentified α chains mentioned above. The laminin $\beta 1$ chain is replaced by the $\beta 2$ chain somewhat later in glomerular development. In general, the integrins found to be expressed in glomeruli of adult kidney were consistent with those found in cultured glomerular visceral epithelial and mesangial cells. No splice variant switch of the integrin $\alpha 3$ or $\alpha 6$ subunits could be demonstrated during nephrogenesis. Our results suggest an important role for the mesangial cell in providing nidogen as a crucial component of the supramolecular structure of the glomerular basement membrane. Furthermore our results indicate that laminin $\alpha x\beta 2\gamma 1$ and $\alpha y\beta 2\gamma 1$ isoforms are important in the glomerulus of adult kidney and that the integrin $\alpha 3A\beta 1$ is the main integrin receptor for laminin isoforms on glomerular visceral epithelial and mesangial cells, both in vitro and in vivo.

AB It has become apparent that extracellular matrix components and their cellular receptors, the integrins, are important regulators of glomerular development and function. In this rapidly evolving field we studied the production of extracellular matrix components and integrins by rat glomerular visceral epithelial and mesangial cells, using molecular probes and antibodies that have recently become available. Special attention was paid to laminin isoforms and to splice variants of the integrin subunits $\alpha 3$ and $\alpha 6$. Results were compared to the in vivo expression in human fetal, newborn and adult kidneys. The mesangial cells were found to produce laminin-1, nidogen and two as yet unidentified laminin isoforms with putative α chains of about 395 (αx) and of 375 kDa

($\alpha\gamma$), tentatively described before as bovine kidney laminin. Furthermore, they expressed the integrins . α 1. β 1, . α 2. β 1, . α 3A. β 1, . α 5. β 1, . α v β 3, α v β 5, and small amounts of α 6A β 1 and α 6B β 1. The glomerular visceral epithelial cells produced the two new laminin isoforms mentioned above, laminin-5, but no laminin-1 or nidogen. The integrins α 2. β 1, . α 3A. β 1, . α 6A β 4, α 6B β 4 and the integrin subunit α v were found to be expressed. We show that during nephrogenesis, the laminin . α 1 chain disappears and is replaced by another α chain, possibly one of the two as yet unidentified α chains mentioned above. The laminin β 1 chain is replaced by the β 2 chain somewhat later in glomerular development. In general, the integrins found to be expressed in glomeruli of adult kidney were consistent with those found in cultured glomerular visceral epithelial and mesangial cells. No splice variant switch of the integrin α 3 or α 6 subunits could be demonstrated during nephrogenesis. Our results suggest an important role for the mesangial cell in. . . glomerular basement membrane. Furthermore our results indicate that laminin α x β 2 γ 1 and α y β 2 γ 1 isoforms are important in the glomerulus of adult kidney and that the integrin α 3A β 1 is the main integrin receptor for laminin isoforms on glomerular visceral epithelial and mesangial cells, both in vitro and in vivo.